Control options of plant pathogens in native plant communities in south-western Australia

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Abstract

Control of plant diseases in natural communities can involve management practices such as hazard rating, hygiene measures, quarantine, chemical applications, plant breeding, biological control agents, and molecular manipulations involving hosts, pathogens and beneficial microorganisms. This paper will examine traditional, immunological and nucleic acid-based methods for the detection, identification and control of plant pathogens and their application in native plant communities.

Introduction

The plant diversity of south-western Australia is unique, with some 9,000 plant species, many of which are endemic. As many as 2000 species may be susceptible to *Phytophthora* species (Wills 1993), and many more are susceptible to other pathogens. Natural ecosystems are important centres of biodiversity and are important assets for tourism, recreation and conservation. The management of plant communities to maintain their intrinsic value includes disease control.

Considerable worldwide research has concentrated on the control of plant diseases; however, the majority of this work concerns economically important crop plants. Crops are usually grown in monoculture on a broad scale under well-fertilised, weed-free, and often, irrigated conditions. The plants are usually selected lines, bred for uniformity in germination, growth, crop maturity, and yield; in addition, cultural conditions are usually reasonably homogeneous. This compares with the huge diversity of plant species, uneven distribution of individuals, genetic variability, soil types and micro- and macro-environmental conditions present in a natural ecosystem.

There are many ecological differences between agricultural and natural ecosystems, and those that set them apart embrace the concepts of population size, density, and spatial distribution, genetic variability in host populations, and population continuity or predictability through time (Burdon 1993). In natural ecosystems, plants are adapted to their pathogens; those that are not adapted are replaced by those better adapted. If a plant becomes unusually plentiful, because of favourable conditions, its parasites increase with it and reduce the fitness and subsequently the number of susceptible plants, which in turn results in a reduction in the numbers of the parasite. The population of an organism in a given ecosystem is under continuous adaptive selection, through interaction with other organisms, for greater fitness (Cook & Baker 1983). It immediately becomes apparent that enormous complexities are involved in applying any control method in a natural, compared to a cultivated, system.

Epidemics of plant disease in natural plant communities are relatively commonplace; examples include outbreaks of *Albugo candida* and *Peronospora parasitica* and their effects on the survival of *Capsella bursa-pastoris* (Alexander & Burdon 1984), and flax rust *Melampsora lini* on wild flax *Linum marginale* (Burdon & Jarosz 1992). The occurrence of a plant disease epidemic in a natural community indicates that some aspect of the ecosystem is not in balance. A number of essential changes need to occur for disease outbreaks to occur (Cook & Baker 1983):

1) the pathogen is genetically homogeneous, (implies introduction) and highly virulent, in high inoculum density or is not in balance with its antagonists;

2) the abiotic environment is relatively more favourable to the pathogen than to the host and/or the antagonists;

3) the host plant is genetically homogeneous, highly susceptible, and continuously or extensively grown (as in cultivated crops); and

4) the antagonists are absent or in low numbers, lack suitable substrate or the proper environment to function as antagonists, or are inhibited by other microorganisms.

Therefore, in cultivated crops plant diseases can be endemic or introduced. In a natural community pathogens are usually introduced or alternatively environmental factors have changed (due to man or nature), that predispose plants to a pathogen. Therefore, it is important to consider in detail the classical disease triangle (host-pathogen-environment), when considering a plant epidemic in a natural ecosystem.

In Western Australia, most of the "natural" ecosystems have been changed in some way, through such influences as forestry, wildflower picking, fire management, mining activities, road building, bushwalkers, clearing farmland and hydrological changes due to many of these activities. These can all affect the biological equilibrium and result in disease outbreaks. It is often hard if not impossible to associate such changes with a disease outbreak, if the pathogen is proven not to be introduced. Therefore, our information base on our

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knowledge of any potential endemic or introduced pathogens needs to be extended. This includes their specific requirements for survival, means of dissemination, host range, infection processes and pathogenicity. These requirements are further influenced by climatic changes (on pathogen and host), by human activities (management, economic and casual), or by fauna activity (defoliators and borers).

It can still be argued that many areas of the biology, ecology and pathology of most if not all pathogens (including *P. cinnamomi*) are not well understood and, until this is so, the appropriateness of many control strategies such as biological and chemical control or burning are questionable. Therefore, the main priority in controlling a pathogen is to have a comprehensive understanding of its biology, ecology, pathology and host range. For example, although many assumptions are made about the importance of chlamydospores and oospores as survival structures, it is still to be conclusively shown for most *Phytophthora* species that these survival structures are produced naturally in soils and plants and, if so, how long they survive and how readily they germinate. Considerable research has been done *in vitro* and *in vivo* on propagules that have been artificially produced on or in rich artificial media and then placed into soils under various temperature and moisture regimes. How these survival structures relate to those that are produced naturally is not known. For example, oospores of *P. citricola* which were produced axenically in V8 broth cultures had very short survival times compared to those produced in non-sterile soil extracts. Those produced in non-sterile soil extracts survived many months (Hardy, *unpublished data*).

In this paper control options have been divided into five main groups cultural, resistance, chemical, biological and molecular strategies.

Cultural control options

Cultural control options aim to restrict the spread and reduce the amount of inoculum. In natural ecosystems such as the forests, heathlands and the banksia woodlands of the south-west, one potential control strategy is manipulation of the environment with fire management. Once the biology and ecology of existing pathogens and the ecology of hosts are known, fire could be used to successfully reduce inoculum levels. However, one consequence of using fire may be to provide infection via wounding by other pathogens, particularly aerial ones. Quarantine measures are also effective in reducing the spread of soil-borne plant pathogens, particularly for introduced pathogens, such as *P. cinnamomi*. For aerial pathogens, quarantine is of little value since they can be spread great distances by wind which cannot be controlled by quarantine. Cultural techniques should be explored for the control of plant pathogens in natural ecosystems, especially for those plants which are endangered.

Resistance

Induced resistance or immunisation

Recently, immunisation (cross protection) against plant disease has been well documented for a range of pathogens in widely diverse plants and plant tissues (Kuc 1990). Plant immunisation has proved successful in expressing, or sensitising for expression, resistance mechanisms in plants which are considered economically important. Induced resistance essentially involves infecting plants with an avirulent isolate of the pathogen, or a non-related pathogen which induces a host resistance response. This in turn effectively stops the invasion of the pathogen in question. The induction of resistance in tobacco to tobacco mosaic virus with specific chemicals such as salicylic acid and methyl-2,6-dichloroisonicotinic acid has been shown (De Waard *et al.* 1993). Such techniques may in the future be useful for the control of certain plant diseases in natural systems, such as the preservation of rare and endangered species.

Plant breeding for resistance

Breeding for resistance in natural ecosystems where there is a large diversity in plant species is not practical, due to associated costs of developing and introducing resistant plants. The only areas where it would be practical would be to preserve specific attributes of rare and endangered flora and in rehabilitation of severely denuded areas (mass collapse sites and rehabilitated mines). This topic is covered by McComb *et al.* (1994).

Chemical control

Chemicals were used to control plant diseases long before their causal agencies were known. They have been effectively used in intensive agronomic situations, but the control of plant pathogens in native plant communities by chemicals is generally not practical due to costs of the chemical(s) and their application. Possibilities of phytotoxicity, resistance, effects on fauna and other beneficial fungi need to be considered. It is possible that the use of fungicides can tip the balance in favour of opportunistic pathogens. In such instances, the use of fungicides at very low rates in conjunction with other factors may be beneficial.

In natural communities, systemic fungicides (which enter the plant, become generally distributed within it, and render the tissues resistant) to attack are the only real chemical option. Most systemic fungicides currently on the market are translocated in the apoplast (Manners 1993); therefore application of systemics is likely to result in their being extensively distributed, via the xylem, throughout the plant. Conversely, application of fungicides to the soil as drenches or seed dressings will not be practical in an extensive and diverse natural environment. However, this should not preclude their application in certain instances or stop further research on their use.

Recently research has shown that the use of neutralised phosphorous acid, which is inexpensive, has low toxicity to plants and animals and has high mobility within plants, will have considerable value in the conservation of rare and endangered plant species (Shearer *et al.* 1991). However, on a broad scale, costs of application, differing tolerances of plant species to this chemical, and side effects on invertebrates and other fungi, need further research. It is possible that frequent use might lead to the selection of resistant pathogenic strains to the chemical (Cohen & Coffey 1986, Coffey 1991), although there is no evidence of resistant strains developing during the last decade of use (Guest & Grant 1991). In addition, *Phytophthora* species differ in their

tolerance or susceptibility to the chemical, which could give a locally competitive advantage to other *Phytophthora* species in the area. For example, *P. megasperma* is relatively insensitive to the chemical (Dercks & Buchenauer 1987), and is present in areas where *P. cinnamomi* also occurs (Bellgard, *pers. comm.*), such as the Fitzgerald River National Park. Therefore, caution needs to be exercised before phosphorous acid is used extensively in an area. In addition, phosphorous acid does not eradicate the pathogen; it activates the plant defence systems which then stop the spread of the pathogen within tissues. Perhaps the best use of phosphorous acid would be its application for the preservation of rare and endangered plant communities. Additional research is required to clearly determine the exact mechanisms involved in disease resistance after the application of phosphorous acid.

Many fungicides have different effects on a fungus depending on the structures it comes into contact with. For example, a number of fungicides sold for the control of *Phytophthora* species have no effect on endogenously dormant oospores of *P. citricola* and *P. megasperma*, whilst hyphae are killed (Hardy *et al. unpublished data*). These oospores could not be induced to germinate in the presence of the fungicides, but would do so once residues of the fungicides had disappeared. It is possible that such effects might occur with other resting structures such as chlamydospores. Therefore, it is necessary to have an understanding of the effects of a particular fungicide on all stages of a fungal life cycle before it can be used with complete confidence.

Improved knowledge of fungal biochemistry should allow a more rational choice of fungicides, and open the way for the appropriate resistant genes to be cloned and their role in metabolism and pathogenicity explored (De Waard *et al.* 1993).

Biological control

Biological control has been defined as "the reduction of the amount of inoculum or disease producing activity of a pathogen accomplished by or through one or more organisms other than man" (Cook & Baker 1983). The mechanisms of biological control can be grouped into two categories: 1) the use of antagonistic microorganisms, either resident or introduced to reduce the pathogens population level; this includes avirulent or hypovirulent individuals or populations within the pathogenic species itself; and 2) protection of plant surfaces against infection, by genetic manipulation of the plant and the use of specific cultural practices (Cook & Baker 1983).

There are three mechanisms by which biological control may operate in the infection court (rhizosphere or phylloplane):

1) parasitism and predation: there is active contact between microorganisms which result in the degradation of hyphal walls or mycophagy of whole propagules;

2) amensalism: the biological control agent produces antibiotics or toxic metabolic by-products which inhibit the growth of the pathogen; and

3) competition: involves two or more microorganisms competing for the same limited resource, such as oxygen, space, nutrients and moisture.

It is very likely that successful antagonists employ more than one of the above strategies.

The major stumbling block for applying a biological control agent in the field is that the environmental niche into which it is being applied is most likely already occupied. Therefore, a niche for the biological agent needs to be established (Powell *et al.* 1990). Formation of a niche for the bioagent can be accomplished in a number of ways: the biological agent

1) could utilise a substrate not currently used by other microorganisms;

2) may be better adapted physiologically for a particular niche than are the microorganisms currently occupying it;

3) produces an antibiotic which through its activity creates a zone of substrate possession around it;

4) the biological agent is applied with or after a physical or chemical treatment that decreases the indigenous microflora; and

5) is added to the environment in such a form that large amounts of new substrate give it a head start over the indigenous microflora.

A successful biological control agent is likely to employ more than one of the above conditions. However, in natural ecosystems, as with the application of chemicals, costs of application will be high. There will be associated problems with how the control agents are applied, and in the case of soil-borne plant pathogens, how they can be incorporated into the soil profile where they will be effective. In addition, selection of biological agent(s) that can survive and function across a broad environmental range (differences in moisture, temperature, pH, fertility, host range, soil types and microenvironments) and plant communities would be difficult. It will be necessary to obtain a good understanding of the host-pathogen-biological control agent-environmental interactions before the biological control agents can successfully be applied in the field. There is a general misconception that a biological control agent should eradicate a pathogen, but even a balanced equilibrium between a pathogen and its antagonist(s) should be considered beneficial.

dsRNA for the control of *Phytophthora* species.

Many fungal species have been found to contain double stranded RNA viral genomes in their cytoplasm. These have assumed a great deal of importance in recent years with the discovery that these elements attenuate the virulence of the fungal species which cause chestnut blight (*Cryphonectria parasitica*) and dutch elm disease (*Ophiostoma ulmi*). In both of these diseases, dsRNA-containing hypovirulent strains are able to protect the host trees against attack by virulent strains. Protection occurs via transmission of the dsRNA element to the attacking virulent strain converting it to a hypovirulent strain.

The dsRNA elements of *C. parasitica* are the best characterised and studied. Hypovirulent isolates of *C. parasitica* contain a number of dsRNA segments (Nuss & Koltin 1990, McDonald & Fulbright 1991). The number of segments, size and sequence homology may vary between strains. Comparison of dsRNA from an American and a European isolate of *C. parasitica* revealed that each contained a large dsRNA of

about 12 kilobases in size, and a number of smaller dsRNAs that were derived from the large dsRNA. The number, size, and concentration of the smaller molecules varied with the strain and stage of growth (Nuss & Koltin 1990). Polypeptide coding sequences occupy only a small part of the element. *Ophiostoma ulmi* isolates contain a specific set of 10 dsRNA segments ranging in size from 0.34 to 3.5 kb which are associated with transmissible hypovirulence. Not all segments may be transmitted, and healthy isolates recovered from a diseased isolate were found not to contain segments 4, 7, and 10 (Nuss & Koltin 1990).

The discovery of these elements in *C. parasitica* and *O. ulmi*, and their association with the hypovirulent state, spurred a search for similar hypovirulent elements in other species of phytopathogenic fungi with the hope that they could be used as biological agents to control disease. dsRNA elements have now been found in both highly and weakly virulent isolates of the wheat pathogen *Gaeumannomyces graminis* var *tritici* (Nuss & Koltin 1990). However in studies with hypovirulent isolates it was found that virulent isolates could segregate out and that these were free of dsRNA elements, whereas the hypovirulent isolates retained the dsRNA. Isolates of the fungus *Helminthosporium victoriae*, which causes oat blight, have been found to contain dsRNA viruses; one is associated with hypovirulence (Nuss & Koltin 1990). Hammar *et al.* (1989) detected a multisegmented dsRNA element in a hypovirulent isolate of *Leucostoma persooni*, and showed that the elimination of these elements restored virulence. In an elegant series of experiments, Sonnenberg & Van Griensven (1991) showed that La France disease in *Agaricus bisporus* is due to the transmission of a multisegmented dsRNA from a diseased to a healthy isolate by hyphal anastomosis. DNA markers were used to show that there was no transmission of either nuclei or mitochondria between the strains.

In spite of the evidence that dsRNA confers hypovirulence to fungal isolates there are instances where they may enhance virulence, or have no effect. Tooley *et al.* (1989) studied the distribution of dsRNA elements in isolates of *Phytophthora infestans* and found that 36% of Mexican isolates contained dsRNA. However there was no correlation between the presence of dsRNA and virulence. Studies with *Rhizoctonia solani* have reached different conclusions. Castanho & Butler (1978) isolated three segments of dsRNA from a diseased isolate of *R. solani*. Healthy isolates recovered from this diseased isolate by hyphal tip culture did not contain dsRNA. In plate tests it was shown that the diseased isolate could protect plants against the healthy isolate. However, Finkler *et al.* (1985) compared virulent and hypovirulent isolates of *R. solani* from Israel, and found that only the virulent isolates contained dsRNA. Transmission of virulence to a hypovirulent strain was found to be associated with transmission of the dsRNA. More recently Bharathan & Tvantzis (1990) found dsRNA in all isolates tested from diverse locations in the USA and Canada. Isolates from 5 anastomosis groups (AG) representing a wide range of virulence were included in the study. There was a high degree of heterogeneity among dsRNA's from the same isolate, or from isolates within the same AG. This was especially evident in AG4, dsRNA's from these isolates were highly specific for the isolate from which they came indicating a lack of horizontal transmission of genetic elements in this AG. Cross hybridisation did occur among dsRNA segments from 3 hypovirulent isolates belonging to AG's 2, 3, and 5, suggesting that there may be a sequence involved in suppression of virulence. However, the results show that overall there is no correlation between the presence of dsRNA and virulence. Similar conclusions were reached in a study of wheat infecting isolates of *R. solani* AG8 in Western Australia (Yang *et al.* 1994).

What are the prospects for the use of dsRNA elements in biocontrol of pathogens such as *Phytophthora*? Theoretically the use of dsRNA elements for biocontrol of *Phytophthora* is possible. The biocontrol agent would colonise the same microhabitat and is subject to the same influences as the pathogen. Moreover, since the antagonist converts the pathogen to an antagonist, the level of control will not decrease (provided that the dsRNA is not debilitating). Studies on the distribution of dsRNA elements suggest that they are widespread, and that there should be no trouble finding them in isolates of *P. cinnamomi*. However, not all of these would be expected to be hypovirulent, and in fact some may be hypervirulent. Hypovirulent elements also have the additional disadvantage that they may debilitate the host strain making it less able to withstand competition from other microflora. This would result in the eventual disappearance of the strain from the environment. The results of previous studies suggest that we would be lucky to find a hypovirulent element which could be effectively used as a biocontrol agent. How do we decide which dsRNA elements we should use? The elements should not severely debilitate the host strain, and should be capable of being transmitted to other strains from the same species despite incompatibility barriers between the strains. The elements should be stably maintained in different genetic backgrounds. Finally, in deciding which elements are hypovirulent it is important to take into account the level of genetic variation between host strains. Hypovirulent factors are identified by comparison of the pathogenicity of dsRNA containing and dsRNA free isolates. However, in this regard it is essential that the isolates being compared are characterised by means of DNA fingerprinting to ensure they are isogenic except for the dsRNA element. The application of DNA fingerprinting techniques has shown that isolates which look and behave the same are often quite different. Finally, if hypovirulence is shown to be an important form of disease control, there are the questions of how will it be introduced into the environment and how long it would take to function? These questions are particularly pertinent considering the huge and diverse areas of vegetation affected.

In the event that we do not identify a suitable dsRNA element for biocontrol, we still have the possibility of using dsRNA elements as delivery vehicles for dominant avirulent genes. These genes could be artificially-constructed antisense versions of pathogenesis genes, or naturally-occurring fungal avirulent genes. Using cloned fungal pathogenesis genes we can construct antisense versions of these genes in the laboratory, and insert these by gene splicing into dsRNA elements (to create a dsRNA*). Transformation of the dsRNA* into a strain of the target organism would create a biocontrol agent. Expression of the antisense version of the pathogenesis gene would inhibit expression of the sense gene thereby attenuating virulence. The dsRNA* would be transmitted to pathogenic strains in the same way as a naturally occurring hypovirulent element, and would convert those virulent strains to hypovirulent strains.

Molecular strategies for disease management

Molecular strategies available for disease control are primarily concerned with crops of agronomic importance. However, the use of molecular strategies of disease control in native plant communities will in the medium to long term provide beneficial tools in the area of disease diagnosis. The use of molecular techniques for early diagnosis, genetic manipulation of the pathogen(s), biocontrol agents and the host(s) hold considerable promise. However, increased funding into these techniques at the expense of traditional plant pathological strategies and ecological studies must not occur. It is imperative that these disciplines occur in conjunction with each other.

Diagnostics

The early and accurate diagnosis of plant diseases using molecular strategies, such as immunological techniques, and nucleic-acid based methods could become an integral management strategy of native plant communities. Management of plant diseases is most effective if control measures can be introduced at an early stage of disease development. Reliance on symptoms is often inadequate in this regard, since symptoms often appear long after disease establishment. Although biological techniques of disease diagnosis are usually very accurate, they are slow and not amenable to large scale applications. Molecular techniques of diagnosis must be viewed as management tools, to be used in conjunction with other diagnostic procedures, knowledge of the host, and an understanding of the ecology of the disease and the biology of the pathogen. For example, a pathogen may be detected in a locality but not cause disease due to one or more of the host, pathogen or environmental factors are not optimal for the disease to occur.

Molecular biology now provides rapid, specific, and sensitive techniques for detection of some plant pathogens. They will in the future become important early diagnostic tools for the early identification of plant pathogens.

Immunoassays in plant pathogen detection

Methods such as serological assays for pathogens, particularly viruses have been available for many years. Immunological assays include enzyme linked immunoabsorbent assays (ELISA), immunofluorescent assays, monoclonal and polyclonal antibody assays. Immunoassays have the potential to detect and quantify pathogen propagules in soil and other substrates. The role of an immunoassay is to reveal the presence of specific complexes between the antibody and antigen, that are unique to the pathogen. Immunological techniques can aid successful plant protection since they permit the early detection and correct identification of important pathogens. As many fungicides are specific only to certain pathogens or groups of pathogens, immunodiagnosis can help in the selection of the most appropriate fungicide treatment (Fox 1993). Immunological techniques can be used to quickly and accurately recognise and identify those pathogens with variable or latent symptoms on the host plant.

Nucleic-acid hybridisation based detection of pathogens

At present the exploitation of nucleic acids, DNA and RNA, in practical methods for the detection and/or identification of plant pathogens is in its infancy, and it will be a number of years before such methods will be of practical benefit. This is in comparison to the use of molecular methods in clinical pathology, or the use of immunological methods in phytopathology (Fox 1993). However, the potential advantages of this technology are overwhelming and it is inevitable that their adoption will be widespread. Nucleic-acid hybridisation depends on the high degree of specificity inherent in the pairing of nucleotide base sequences. This specificity allows the technique to be used for diagnostic purposes. The detection of plant pathogens via their nucleic-acids has two major advantages over rival technologies (Fox 1993). Firstly, all viable propagules (virus particles, spores, mycelium, *etc.*) contain the entire nucleic-acid complement of the organism. The presence of the nucleic-acid sequences is not altered by development or by response to environment or by the host. Antigens, in contrast may only be present at certain points in an organisms life cycle. In addition, the ability of the polymerase chain reaction (PCR) to detect one molecule of a particular sequence, conveys the ability to detect just one viable cell of the pathogen. This ultimate level of sensitivity obviates the need to culture pathogens prior to identification. Secondly, the identity of an organism is the direct result of the expression of its nucleic acids into protein and RNAs. Thus detection of a nucleic-acid sequence is simultaneously a positive identification. The nucleic-acid sequences in pathogens vary in their homology to sequences in other organisms. Thus it is possible to use nucleic-acid based methods for different levels of discrimination. For example, probes could in theory be designed which detect all fungi, or all ascomycetes, or all powdery mildews, or barley mildew or a particular pathotype in which one might be interested (Fox 1993).

Despite these positive features, nucleic-acid based methods are viewed with suspicion by many plant pathologists. They believe the methods are expensive, complex, slow and involve hazardous chemicals. It is therefore a challenge to plant pathologists to develop cheap, reliable methods suited to routine laboratories and even the end user (forester, farmer *etc.*). The techniques available include dot-blot assays, non-radioactive labels, restriction fragment length polymorphisms (RFLP's), nucleic-acid probes, cloned probes and synthetic probes.

The polymerase chain reaction (PCR)

This is a comparatively new method which relies on two specific DNA primers, a thermostable DNA polymerase and temperature cycling to amplify discrete regions of DNA. It is extremely sensitive with the theoretical potential to detect a single target molecule in a complex mixture without using radioactive probes; and it is rapid and versatile (Henson & French 1993). Unlike serology, synthesis of hundreds of different PCR primers generates costs comparable to those of developing only a few monoclonal antibodies. PCR is capable of quantifying relative differences as well as absolute amounts of scarce target DNA or RNA sequences. The quantification of plant pathogens in diseased plants is possible, since changes of inoculum levels in soil or plants can be monitored by PCR. This can help predict the potential severity of the pathogen and assist in control decisions.

PCR has considerable potential in epidemiological studies. It has the ability to be applied to studying disease resistance and determining at what stage of pathogenesis a

pathogen is inhibited. PCR can be used to estimate the biomass of unculturable microorganisms or obligate biotrophs. Microbial detection methods can be improved by combining PCR with antibody binding, this also gives a better indication of microbial viability. PCR is already being used to advance studies of host-pathogen interactions, such as for *Erwinia* (Blakemore *et al.* 1992), *Pyrenophora* (Reeves & Ball 1991) and *Leptosphaeria maculans* (Goodwin & Annis 1991). PCR could be used to construct pathogen genomic or cDNA libraries, or could be used to construct libraries of host or pathogen genes that are differentially expressed during the infection process.

As PCR methods for detection of pathogens become available, it will be possible to focus research on studying pathogen populations, biology, ecology, variability and host-pathogen interactions (Henson *et al.* 1993). An effective diagnostic test must be simple, accurate, rapid and safe to perform, yet sensitive to avoid 'false positives'.

Development of resistant plants by genetic engineering

In recent years plants resistant to viral diseases have been developed by genetic engineering. This has been achieved by inserting viral genes into the plant genome so that their expression inhibits the normal viral life cycle. This same strategy can be applied to a wide variety of plant viruses. Fungi are much more complex, and use a wider variety of mechanisms to achieve their colonisation of the host plant. Nonetheless we are beginning to identify the mechanisms used by fungi to infect plants. Once we have identified these mechanisms we can engineer the plant to these and thus confer resistance to these plants.

One approach is to use fungal inhibitory proteins. This does not depend on knowing the mechanism of infection. Genes for the proteins are inserted into the plant genome where they are expressed. In two separate studies plants resistant to *Rhizoctonia solani* have been engineered by inserting the chitinase gene from bean (Broglie & Broglie 1993), and the barley ribosome inhibiting protein gene (Logemann *et al.* 1992) into the plant genome. Both of these mechanisms on their own conferred higher levels of resistance to *R. solani*. These studies demonstrate the utility of the general approach of using antifungal proteins to engineer resistant plants. Many plant species contain antifungal proteins, be they lectins which bind to the fungal cell wall and inhibit growth, or enzymes which degrade the fungal wall.

Another approach would be to inhibit the enzymes produced by the pathogen and which are necessary for infection (Kotoujansky 1987). Many soft rot pathogens such as *Erwinia* produce pectic enzymes which breakdown the pectic substances producing the typical soft rot symptoms. Most of these enzymes produce oligogalacturonide degradation products which induce a defence response in the plant and thereby limit infection. Highly virulent isolates produce additional compounds which degrade these elicitors and prevent induction of the plant defence responses. These enzymes can be targeted by the use of polygalacturonase inhibiting proteins (PGiP) which have been described in all dicot species (Hoffman & Turner 1984). Potentially, by transferring the gene for such an inhibitor to the plant genome, the inhibitor would prevent colonisation by highly virulent strains.

A variation on this theme would be to use antibodies, or more correctly plantibodies. A number of studies have now demonstrated that we can now synthesise antibodies in plants (Pluclzltun 1992). This can be achieved by transformation with antibody synthesising genes. Antibodies against fungal extracellular enzymes or against the fungal wall components could inhibit infection of the host. Similarly, antibodies against detoxifying enzymes could be used to achieve resistance. The fungus *Nectria haematococca* is a pathogen of peas. The fungus produces the enzyme pisatin demethylase which inactivates the phytoalexin pisatin produced by the host (Schafer *et al.* 1989). The production of plantibodies against this enzyme would enable the host to limit colonisation by the fungus.

Genetic engineering of resistant plants can be achieved by using genes derived from the pathogen. Incompatible reactions between the pathogen and the host are determined by avirulent (avr) genes in the pathogen which act with genes in the host to induce defence responses and limit the spread of the pathogen. One novel idea suggested is to take the avr gene and place it in the plant genome by transformation technology (DeWitt 1992). The gene is modified in such a way that any infection would trigger it's expression. This in turn would induce expression of the host defence responses. Thus instead of the pathogen carrying the avr gene, the host would carry it and it could protect against highly virulent isolates.

The phenomenon of induced resistance offers great potential for engineering resistance against a range of fungal pathogens. This is a non-specific resistance induced by infection of the plant. For example, the infection of the lower leaves of tobacco with TMV leads to resistance against fungi, viruses, bacteria, and insects. Concomitant with this resistance a large number of proteins are synthesised within the plant, these include glucanases and chitinases (Garner *et al.* 1992). Resistance against *Phytophthora infestans* has been achieved by this mechanism. Potentially if we can identify the genes involved in the induced resistance we can develop strategies to turn on these responses rapidly in the event of an infection.

The defence responses in the host include activation of lignin and phytoalexin biosynthesis. The level of phytoalexins may be modified to confer resistance to fungal pathogens. It has been found that the activity of the enzyme isoflavone-2-hydroxylase regulates the amount of phytoalexin synthesis in chickpea cell suspensions. Increasing the activity conferred resistance to *Ascochyta pisi* (Lamb *et al.* 1992). Greater levels of resistance could be achieved by modification of the gene for increased expression.

Plants can be modified to produce new types of phytoalexins, thus conferring resistance against normally pathogenic fungal species. The phytoalexin resveratrol is synthesised by stilbene synthase in a single step from p-coumaroyl CoA and malonyl-CoA (Lamb *et al.* 1992). Introduction of the stilbene synthase gene into tobacco caused the synthesis of resveratrol in tobacco.

There are now a number of strategies emerging for the genetic engineering of plants resistant to fungal diseases. The impetus for this work is the lack of alternative control measures. In many cases there are simply no natural sources

of resistance, and no effective fungicide treatments for control of the pathogen.

Integrated control

Integrated control will become increasingly important, especially as our understanding of interactions between host plant, pathogen, biological control and environment improves. However, the range of strategies available for integrated control in native plant communities are not as diverse as those in broad acre agriculture or horticulture. It is possible that an advantage of integrated control is the synergistic effect of combining practices (Coffey 1991). In intensive agriculture, many of the success stories of pathogen control include the incorporation of breeding strategies and chemicals, cultural and biological applications. However, integrated control is not a panacea for pest and disease control but an ecological approach to maintaining plant health (Kendrick 1988).

Existing control strategies of pathogens in natural plant communities in south-western Australia

Phytophthora species

The main control strategies for *Phytophthora* species in Western Australia have centered on *P. cinnamomi* and include hazard rating, assessment of risk, hygiene and quarantine measures, unfavourable to the pathogen but enhancing host resistance (Shearer & Tippett 1989). Hygiene includes planning, training, and exclusion methods such as strategic road placement, washdown facilities between infected and non-infected sites, confining activities such as logging to periods of least risk and strategic road management (Shearer & Tippett 1989). Management strategies which are unfavourable to the pathogen can also be effective. These include the manipulation of understorey by reducing *Banksia grandis* by fire or stump poisoning which reduces a potential source of inoculum. Fire can also stimulate *Acacia* growth, specifically *A. pulchella,* which has been shown to suppress *P. cinnamomi* activity. Chemical and biological control can also be used and have been discussed previously.

Until it is clearly established whether other *Phytophthora* species are endemic or not, strategies used for the control of *P. cinnamomi* may or may not be effective for these other species. Currently, detailed studies are being undertaken on *P. citricola* (F Bunny, *pers. comm.*) and *P. megasperma* (S Bellgard, *pers. comm.*). Both these species are homothallic and undergo sexual recombination which can add additional complexities to control compared to *P. cinnamomi* which is heterothallic. *P. citricola* is most frequently isolated from areas of disturbance such as mine rehabilitation, log landings and drainage lines (F Bunny, *pers. comm.*). Oospores have always been assumed to be important survival structures formed in soil and host plants. However, it is only recently that they have been shown to be produced in nonsterile soils. Oospores are now known to survive for a minimum of months in field soils which indicates their importance as survival structures. It is now almost certain that *P. citricola* is endemic in the jarrah forest (F Bunny, *pers. comm.*), and this is likely to be true for other areas of the south-west. Therefore, it is extremely important to increase research into the ecology and pathology of *P. cinnamomi.*

The importance of *Phytophthora* species as damping-off pathogens in natural ecosystems must not be discounted. Recently, it has been shown that *P. citricola* can behave as a post-emergent damping-off pathogen in mine rehabilitation (Woodman 1993). Therefore, additional research is required to ascertain whether *P. citricola* and other *Phytophthora* species can cause damping-off, especially in areas of disturbance (drainage lines, road verges, salt effected, waterlogged, fire damaged).

It is also important to consider that the ecology and pathology of a pathogen may differ in different environments. This has been shown with *P. cinnamomi* in rehabilitated mines. The mining process changes the soil environment substantially. Recently, extensive excavations of 1-6 year old *E. marginata* growing in rehabilitated mines and exhibiting early symptoms of infection have clearly shown that *P. cinnamomi* infects the collar and lignotuber region in preference to roots. With time the pathogen moves down into the roots and up the stem. Over 30 jarrah trees have been excavated, and at no time has *P. cinnamomi* been isolated from roots of trees exhibiting symptoms of early infection (Hardy *et al. unpublished data*).

The eradication of *Phytophthora* infections on woody plants by the use of chemicals has been shown to be almost impossible on plants growing in infected soils or container mixes, due to the formation of resistant survival structures such as chlamydospores and oospores (Ribeiro *et al.* 1991). In addition, a range of *Phytophthora* species, including *P. cinnamomi* and *P. megasperma* in plantations of *Abies procera, Pseudotsuga menziesii, A. magnifica* var. *shastensis* and *A. grandis* in the north-western United States could not be effectively controlled (Ribeiro *et al.* 1991). In contrast, infection of apple trees with *P. cactorum* could be eradicated / cured with the systemic fungicides metalaxyl and fosetyl-Al however, repeated applications were required (Ellis *et al.* 1986, Orlikowski *et al.* 1986). Therefore, fungicide application in natural ecosystems is unlikely to be effective in the long term, even if the costs of the fungicides and their application is not taken into account.

Stem and branch cankers

The distribution of canker fungi has mainly been ignored in all natural environments of the lower south-west. This is despite the observed increased incidence since the 1970's of eucalypt die-back decline (Kimber 1980). A number of pathogenic fungi have been associated with cankers of stem and branches of forest trees in south-western Australia (Davison & Tay 1983), and in heathlands and woodlands (Murray *et al. unpub. obs.*). Recent work on *Diplodina* (anamorph, *Cryptodiaporthe*) canker on *Banksia coccinea* suggests that this fungus is endemic (Shearer 1994) The outbreak of this fungus as a major pathogen indicates that some change in the biological balance of the system has changed. Schoeneweiss (1975) has associated disease caused by canker fungi to be aggravated by transient stress factors, such as excessive heat or waterlogging.

Murray, Wills & Hardy (*unpublished data*) examined 1259 cankers on 508 native plants, 49 different genera of fungi were isolated, of which putative pathogens included *Botryosphaeria ribis*, *Diplodia mutila* (teleomorph *Botryosphaeria stevensii*), *Endothiella* and a species of *Diplodina*. However,

B. ribis and *D. mutila* were isolated respectively, from 53 plant species in 24 genera and 23 species in 13 genera, which indicates their broad host range. In addition, a wide range of fungi were isolated from stem and branch cankers of plants grown on rehabilitated mines (Carswell 1993). Pathogenicity testing proved many of these to be pathogenic. It is likely that conditions in rehabilitated or other severely denuded sites (lack of canopy cover, high or low moisture levels and reduced plant diversity) could predispose plants to canker or other pathogenic fungi.

Armillaria luteobubalina.

Armillaria luteobubalina is a primary pathogen widely distributed throughout the south-western Australia; it is a native pathogen that infects a wide range of plant species from diverse families (Pearce *et al.* 1986; Shearer & Tippett 1988). It has been isolated from wandoo, jarrah, and karri forests as well as throughout the coastal fringe from Cape Arid up to Cervantes (Shearer *et al.* 1994). In the jarrah forest, the impact of this pathogen varies between plant community and climatic zone (Shearer & Tippett 1988). Control has been effective in certain instances with other wood rotting fungi such as *Coriolus versicolor* and *Stereum hirsutum* (Pearce & Malajczuk 1990, Pearce 1990). These wood decay fungi were shown to significantly reduce the invasion of *Eucalyptus diversicolor* stumps by *A. luteobubalina*. However, there are at present fewer options for the control of *A. luteobubalina* than those available for *Phytophthora*.

Conclusions

It is necessary for extensive and detailed surveys to be initiated to increase our knowledge of the identity and incidence of pathogens causing disease in the natural plant communities of the south-west of Western Australia. Detailed studies of pathogen survival, reproduction and spread as well as host infection and susceptibility need to be undertaken. Each pathogen and its interactions with each of its hosts must be considered individually. In turn, these need to be related to interacting factors such as environmental changes, insect associations and the influence of human activities. A comprehensive understanding of the pathogens present, their life cycles and how they are influenced by environmental and human interactions will help ensure that our management of these native ecosystems is effective. Consideration must be made to ensure that management practices do not consider a few pathogens to the exclusion of others.

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